Use of Escherichia coli genetically modified as a cooper biosensor for educational and research purposes

Risk assessment of Zamorano iGEM´s project

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SERVICIO NACIONAL DE SANIDAD AGROPECUARIA (SENASA)

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Evaluation Purpose

To introduce the new organism has 2 goals:

A) Educational: As a laboratory module for students from Zamorano University on the learning by doing program. The learning objectives include to build student capabilities on basic bacteriology, aseptic techniques, general principles of biosafety inside the lab, general principles of genetics and molecular biology and to introduce the students on topics of genetic engineering and into synthetic biology as a cutting edge applied science.

B) Research: Biosensor detector of heavy metals in water, as a biological technique to indicate the presence of cooper traces in water due to the abuse of agrochemicals. A mechanism of detection it's the first step for the managing and responsible use of such compounds and the preservation of water resources in a specific area.

Booth objectives were established due to the feasibility of its usage. The bacterium that changes color in presence of cooper becomes a biosensor to detect contamination caused by this metal.

For the developing of the project, there were used bacteria *Escherichia coli* from the non-pathogenic strain DH5ΔZ1. It was added a plasmid with protein producing genes that changes the bacteria color in presence of cooper.

To achieve this objective it was necessary to insert specific promoters for the expression pigmentation genes. As a molecular

marker used to detect transformed bacteria, it was used standard antibiotic resistance genes specifically for ampicillin and tetracycline.

Genetic material description

As detailed before, the organism used for the project *"Construction of bioscience knowledge"* was a non-pathogenic *E. coli* of the DH5ΔZ1 strain whose genome was intact.

To achieve the pigmentation change on the bacteria, it was used the plasmid 5006 pd, derived from the pJ8004 plasmid (Figure 1), synthetized by the company DNA 2.0. This plasmid provides resistance to two antibiotics ampicillin (AmpR+) and tetracycline (TerR+) on lab conditions (See annex 1).

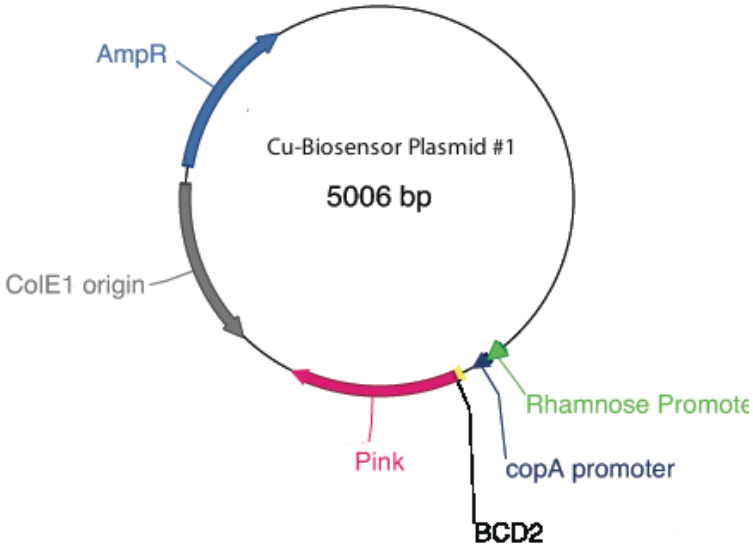

Figure 1. Description of the genetic construction showing a pink pigmentation gene

The plasmid contains: Replication origin site (ColE1 origin), ampicillin (AmpR+) and tetracycline (TetR+) resistance gene, cooper detection sequence (CopA promoter), pigmentation gene (pink, green or fluorescent orange), Rhamnose promotor (F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF, U169, hsdR17(rK- mK+), λ−, LacIq+.).

The replication origin site ColE1 present in the plasmid allows approximately 15 to 20 copies of the plasmid for each cell. (copy average).

The bacteria developed with naturally in the absence of cooper, with no phenotypic changes detected in the organism. Once the modified bacterium was in contact with cooper diluted in water, the color changing reaction manifested depending on the pigmentation gene inserted (pink, green or fluorescent orange).

SYSTEM USED TO PRODUCE THE REGULATED ARTICLE

The pink color derives from the registered gene with trademark CupidPink https://www.dna20.com/products/protein-paintbox?exp=2 # collapse10 from the company DNA 2.0.

The green color derives from the registered gene with trademark https://www.dna20.com/products/protein-paintbox?hl=gfp#collapse27 from the company DNA 2.0.

The orange color derives from the registered gene with trademark YukonOFP https://www.dna20.com/products/protein-paintbox?hl=gfp#collapse44 from the company DNA 2.0.

The gene reactions are under the control of two promoters: the CupidPink gene uses rhaPBad (ramnose active promoter) and the COPA promoter (inducted by cooper), booth are necessary for the reaction to take place. The gene it's under a constitutive promoter so it is always expressed as long as there is metal present.

Transformation methods

The genetic transformation of the DH5ΔZ1 strain was achieved in collaboration with the Endy Lab and Stanford University, Palo Alto, California, EEUU. The genetic material was imported to Zamorano University, Honduras, under an import permit emitted by SENASA from the Honduran Secretary of Agriculture on May 2013.

A standard protocol of bacterial transformation was used with viable cells for a lab exercise with students. The organism practices were conducted in the Zamorano University facilities.

In the annex 2, its detailed information about the transformation method and how the biosensor works.

Evaluation location

Zamorano University, Honduras, Environmental and Development Research Lab.

Biosafety measurements

The bacteria used it's a biosecurity Level 1 (this category demands less restrictive biosecurity measurements). To guarantee the students and the personnel safety who worked on the modification and test of the bacteria, the following biosecurity were taken:

Access:

Only the authorized personnel was allowed entrance to the laboratory.

The laboratory doors remained closed during the experiment.

It was not allowed the entrance to kids or animals on the laboratory facilities.

Personnel protection:

1. At all times there were used lab coats while working on the lab.
2. There were used protection gloves for all the procedures that may involve direct or accidental contact with blood, body fluids and any other potentially infectious materials. Once used the gloves were removed aseptically followed by a hand washing.
3. All personnel had to wash their hands after handling lab material, as well as when leaving the working area.
4. There were used security goggles, face protectors and other devices when needed to protect the eyes from spilling, impacts or UV radiation.
5. The laboratory jackets and the rest of the protection equipment were exclusively used for authorized areas inside the lab in order to maintain the aseptic integrity of the practice.
6. There were used toe protected shoes.
7. In the working areas there was forbidden to eat, drink, smoke, use of make up or handling contact glasses.
8. It was forbidden to keep food or drinks for human consumption in the working areas of the lab.
9. The protection clothing was stored on a different storage than the regular use clothing.

Procedures:

1. It was strictly forbidden to create vacuum during pipetting using the mouth.
2. There was no material in contact with the mouth.

3. All technical procedures were conducted in order to reduce at minimum the formation of aerosols and fine drops.
4. It was mandatory to let the lab supervisor know in case of a spill, accident or real exposition or potential to infectious materials. During the development of the project, none of the situations cited above occurred.
5. All contaminated fluids (chemically or physically) were decontaminated before sent to the sewer. Zamorano University has its own treatment mechanism for waste water flow inside the campus as detailed in the bio-contention normative.
6. The written documents planned to go out from the lab, were protected from contamination while in the lab.

For further information regarding the procedure, please check annex 2 (Good aseptic/pipetting practices).

Programed destination

The bacterium was handled completely isolated inside the lab. It was developed on solid medium using petri dishes with nutritious agar and liquid medium using controlled growth on solutions.

During storage the bacteria was keep on exclusively and special refrigerators, hermetically closed in temperatures from 5-8 C° with 45-50% of humidity so the bacteria can enter in a controlled latency status.at he moment of use, there were removed and exposed to the lab conditions (25C°, 50-70% humidity) during a few moments, just to extract the samples and then put back on cold storage in the same medium.

The lab is innocuous, there was not detected the presence of animals or insects that may consist of contamination vectors and spreading of the bacteria. For its manipulation it was required the use of gloves and tweezers to avoid direct contact between the investigator or the student with the bacteria.

Containtment measurements

1. The laboratory was clean and free of non-related to the experiment materials at all times.
2. In the eventuality of accidental spill of potentially dangerous materials the procedure to follow was the decontamination of the area using alcohol and chloride. There were no incidents as mentioned during the experiment.
3. All the instruments, samples and growth medium containing biologic material must have been decontaminated before being

discarded. I the need of re use the material; it was clean and previously sterilized.

4. The windows that could be opened were equipped with filters making impossible the access or arthropods.

Final disposition method

Once the experimental procedures with the bacteria in study were concluded, this ideal environment for the developed organism was destroyed adding isopropyl alcohol to the probing tubes and petri dishes that were used. The addition of the compound killed all the microorganisms present on the growth medium due to cell membrane disintegration and consequent denaturalization of proteins inside them.

After this all waste where put together in special containers separating the liquid waste from the solid waste. Next there were eliminated along with the University's medic clinic waste.

Finally all the instruments used were sanitized with chlorine water and brushes to maintain the asepsis inside the lab. Then the instruments were exposed to high temperatures and pressure inside the autoclave.

The correct handling of bio-waste through applied bio contention procedures was one of the key factors to ensure the fulfilling of the biosecurity norms established and the integrity of all participants. In annex 4 there is more information about the handling of biological waste.

Annex 1

How does a biosensor based on a microorganism works?

The plasmids are widely used on molecular biology for transformations on classic biotechnology and also in synthetic biology. In classic biotechnology genes form a living being are inserted with a sequence already existing in nature. In synthetic biology, the plasmids can also be used as genetic vectors to introduce fragments of artificial DNA (artificial genes) synthetized from chemical compounds (nucleotides A, C, G and T) in a lab. The information from these synthetic genes it's obtained from bioinformatics data and also from the creativity of a gen designer. In synthetic biology "standard parts" are used following the basic principles of engineering. With these artificial or synthetic genes, microorganism genomes can be programed to have a metabolism and function different from natural microorganisms. The transformation technique with a plasmid it's the same in classic bibotechn9ology as in synthetic biology.

Annex 2

Good aseptic practices.

Bacteria are everywhere. Microbiology it's a field of biological science with applications in many fields such as human medicine and veterinary, agriculture, environmental science, industry and bioenergy production. Learning how to properly work applying asepsis it's the basis of microbiology.

To avoid the contamination of Petri dishes and probing tubes:

1. Team learned how to autoclave equipment and reagent as well as growing media.
2. Minimize the time lab equipment exposed to external air.
3. It was used sterilized equipment and surfaces (by fire, autoclaving or chloride).
4. If sterile equipment was set in contact with a non-sterilized surface it was discarded immediately.
5. It was used a new pipette tip every time.

Good pipetting techniques

1. Push softly trough the pipet's end.
2. Push the plunger until the stop position #1.
3. Insert the tip on the liquid.

4. Softly let go the plunger to the initial position.
5. Take the tip out form the liquid and make sure there is no liquid outside the tip nor bubbles inside the tip.
6. Insert the tip in the probing tube or the deposit where the liquid is going to be.
7. Push the plunger to stop position # 1. (Most of the content may come out).
8. Continue pushing the plunger to stop position # 2 (blow out).
9. Take of the tip from the liquid and softly let the plunger go until its original position.

Annex 3

Biological waste management

Even if the *E. coli* strain used for this project requires the biosafety level number 1, the biological waste will be handled as if it was the highest biosafety level (4) to illustrate and learn the biological compounds waste practices.

Note: The biosafety scale goes from 2 to 4.

As an example: The Ebola virus has a biosafety level of 4 and requires laboratories, equipment and highly specialized personnel for its management. The tomato mosaic virus has a biosafety level of 1 for humans (since it is no pathogenic) but the skin contact must be avoided if the person has tomato related activities.

It is not necessary to use neither lab coat nor gloves in this lab.

It is not allowed the consumption of food or drinks in the lab.

Avoid to take objects to the mouth, such as pencils or fingers.

Do not take biological material outside the lab.

Immediately inform the personnel on charge of the lab about any accident or chemical reactive spill in the lab.

Handle the bacterial culture with caution, avoiding placing bacteriological loops or pipette tips on the lab seat. Sterilize bacteriological loops using fire and discard the pipette tips or any other disposable object that may have been in contact with bacterial culture.

Use autoclave ready bags for biological waste such as petri dishes and other materials like pipette tips, and Eppendorf tubes.

Autoclave biological waste in specialized autoclaving bags.

Clean all surfaces on the work area using disinfectant.

Add chloride in the liquid bacterial cultures when no longer in use, if not possible, the procedure is to autoclave the growing media and the glassware immediately.

Carefully wash the hands with water and soap when the practice it’s done.

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